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*APPLICATION OF THE ALTMANN FREEZING-DRYING TECHNIQUE TO PLANT CYTOLOGY**

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Determination of the disposition, quantity and character of mineral elements in plant cells as revealed by the microincineration method of Policard[1] should yield as significant results as have recently been obtained in microincineration studies of animal tissues by Scott[2] and others. This matter has been under investigation here and the initial problem to be solved was the obtaining of an adequate killing and fixing agent. Obviously it cannot contain mineral salts and in their absence the subsequent cytological picture of most plant tissues is poor. In the expectation that it might meet the need of a proper pretreatment for microincineration, the freezing-drying technique of Altmann,[3] which is proving effective in cytological studies of animal material (Bensley,[4] Bensley and Gersh[5]) has been applied to plant tissue. The character of the fixation is as yet not as uniformly good as could be desired, but the cytological results are in some cases so significant that a preliminary announcement of them and a description of an improved type of freezing-drying apparatus is called for.

The Altmann technique consists in killing in liquid air and dehydrating the frozen tissue in a vacuum at a temperature sufficiently low so that appreciable diffusion or displacement of cell constituents cannot occur. An essential feature of our freezing-drying apparatus is an inexpensive refrigeration unit capable of operating at $-30°$C. or lower, yet inviting in its operation a minimum of danger and attention. For this purpose a commercial unit charged with methyl chloride was selected. At $-30°$C. the suction pressure is less than atmospheric so that it is imperative that all connections be vacuum tight. A one-third horse-power induction motor coupled with a 2-cylinder compressor (air-cooled condenser), and running about half-time, maintains at $-35°$C. the insulated system shown in figure 1. Through a thermostatic expansion valve the unit connects (at *A*) with some 16 meters of 1.6 cm. copper tubing whose coils lie in the brine tank (*W*). The calcium chloride brine is continuously circulated by propeller blades driven by the vacuum pump motor (*M*).

High operating efficiency requires proper installation of good quality insulation. The hatched portions of figure 1 represent layers of corkboard cemented together with hot asphalt. An external moisture-proof coating, preferably "trowel" asphalt and sand, is essential. Granulated cork fills the space between the brine tank and the corkboard. The simple vacuum system consists of a Cenco Hyvac oil pump (*V*), the necessary stopcocks (*H*), the drying tube (*F*) containing phosphorus pentoxide, and the tube (*T*) for holding the experimental material. Pyrex glass is used throughout and also standard taper interchangeable ground glass joints.

Comparison of the apparatus shown in figure 1 with that of Gersh[6]

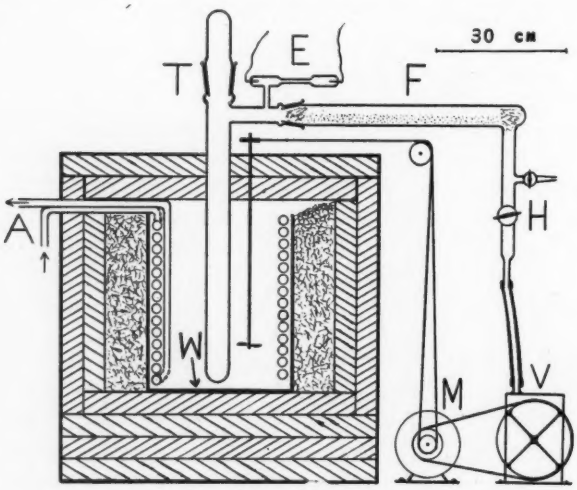

FIGURE 1

Vertical cross-section of the improved freezing-drying apparatus, drawn approximately to scale shown. The refrigerating unit attaches to the coils at *A*.

shows the absence in our apparatus of the McLeod gauge, mercury diffusion pump and embedding chamber. In place of the last a small vacuum bell jar in a paraffin oven has been found to be an effective substitute. Since a McLeod gauge in a water vapor system is not reliable, it has been replaced with a discharge tube (*E*) operated from the secondary of an induction coil (Ford coil).

Certain theoretical considerations involved in the freezing-drying technique and in the construction of apparatus to accomplish it should be noted. In the first place, it is of interest to recall the variation in density

of water as it changes from a liquid at 20°C. (d = 0.998) to ice at 0°C. (d = 0.917), then to ice at −30°C. (d = 0.921). The former transition results in a decrease in density of 8 per cent while the latter reduces the net change to 7.7 per cent. No appreciable increase in density by further cooling is possible (d = 0.930 at −189°C.). This volume expansion would thus correspond to a maximum linear expansion in cell dimensions of 2.5 per cent, assuming a cell to be pure water. Except in the case of tissues with unusually high water content, the probable linear expansion of the cell during the freezing-drying process is only about 1 per cent or a visually imperceptible amount. In the second place, the theoretically correct dehydration temperature must be below the eutectic point of salts in solution and is therefore a variable dependent on the character and physiological condition of the tissue. Scott[2] holds that drying should proceed at or below −54.9°C., the eutectic point of aqueous calcium chloride solution, while Altmann[3] and Bensley and Gersh[4,5,6] report satisfactory fixation of animal tissue at a maximum of −20°C. Between −20°C. and −60°C. there is a decrease in the vapor pressure of a free ice surface from 787 to 8 microns of mercury, a ratio of approximately one hundred. Since the dehydration time increases in the same proportion, routine fixations below about −30°C. would not seem to be feasible except for extremely thin sections of tissue. Whether or not the quality of fixation improves as the drying temperature is lowered still further has not been satisfactorily demonstrated experimentally.

Finally, the factors having to do with the dehydration of frozen tissue and involved in the construction of apparatus to produce it, must be considered. The rate of loss of water vapor from a surface into a perfect vacuum, according to the kinetic theory, is given by

$$m = \alpha P \left(\frac{M}{2\pi RT}\right)^{1/2} \text{grams/cm.}^2\text{/sec.}$$

where m is the mass in grams, M the gram molecular weight, R the gas constant, T the absolute temperature, P the saturated vapor pressure in dynes per cm.^{2} and α a constant. If vapor is present above an ice surface at a pressure of P_v, the resultant loss of ice is given by

$$m = 14.63\ \alpha \frac{P_s - P_v}{T^{1/2}} \text{grams/cm.}^2\text{/min.}$$

where α represents the fraction of vapor molecules which recondenses on the ice surface and both P_v and the saturated vapor pressure P_s are measured in mm. of mercury. For a water surface, Alty[7] has shown α to be approximately 0.01, but its value for ice is unknown. If P_s is 292 microns (its value at −30°C.), then nothing significant is gained by having P_v less than one micron, for their *difference* would not be appreciably

increased even though P_v were equal to zero. At a pressure of one micron, attainable with a rotary oil pump, the mean free path of a water molecule is over 5 cm. Since the radius of the tube holding the frozen tissue is only 2.5 cm., this means that a molecule escaping from the experimental material need only travel one-half its mean free path in order to be taken up by the phosphorus pentoxide which covers the walls of the tube T (Fig. 1). The vapor pressure of phosphorus pentoxide is of the order of 10^{-9} mm. of mercury. Two other processes influence the speed of dehydration. One is the rate of diffusion of a gas through tissue; the other is the speed with which these molecules are removed from the surface of the tissue by the pumping mechanism. It is to be noted that the quantity of vapor transferred per unit time in both cases is proportional to the differences of pressure existing between the points in question. As far as we are aware, no experimental determination of the relative speeds of dehydration as a function of pressure has been made. It is possible, of course, that some unknown factor may influence the results to be expected from the above considerations.

The cytological results briefly summarized below were obtained from tissue treated according to the following procedure (cf. Gersh[6]). Prepared as rapidly as possible, the material was placed in small pill-box containers of monel wire screen which were immersed, as fast as ready, in liquid air where they remained for five minutes. All the containers were removed simultaneously and were lowered at once into the cold dehydrating chamber (Fig. 1, T) and the vacuum immediately established. Various periods of dehydration in vacuo were employed at approximately $-22°$C. and $-32°$C. These were the brine temperatures and, presumably, within the containers a temperature one or two degrees lower was maintained. At $-22°$C. a pressurestat operated by the suction pressure in the refrigerating coils regulated the temperature; at $-32°$C. a mercury thermostat was necessary to restrict temperature variations to $\pm 0.5°$C. At the conclusion of the drying period the material was allowed to come to room temperature in vacuo by lifting the glass system out of the brine tank. Infiltration in vacuo with paraffin was at once begun and was considered to be complete when the material sank. In certain cases the material was transferred to absolute alcohol, absolute alcohol followed by xylol or to xylol alone prior to paraffin infiltration. Treatment with paraffin oil before paraffin, and denaturing by heat were also employed. Sections cut at 5μ became badly split and even disintegrated when an attempt was made to flatten them in the conventional manner on water over an albumen coated slide. It was necessary to cut the sections as flat as possible and spread them with heat on the coated slide without water or to replace water with absolute alcohol. Staining with Heidenhain's iron-alum-haematoxylin proved most satisfactory.

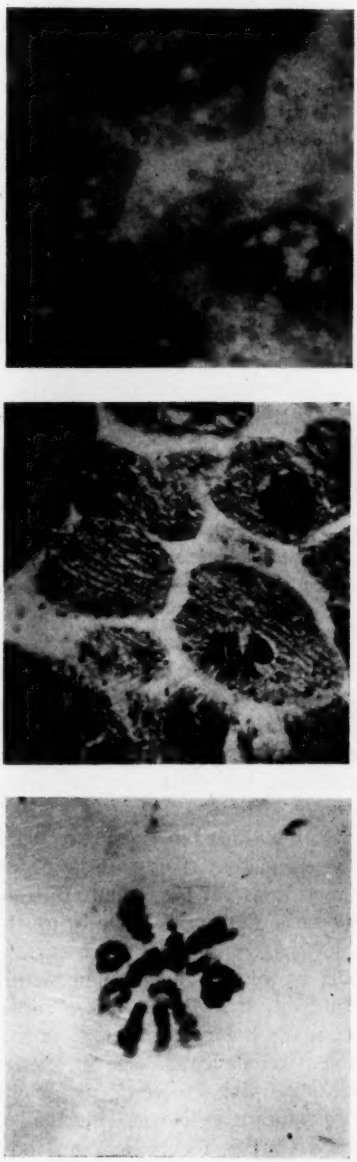

FIGURE 2

To determine whether our apparatus would give fixations comparable in quality to those reported by Bensley and Gersh, thin slices of rat liver, kidney and testis were treated for 14 days at −32°C. Figure 2*a* shows the type of result obtained and comparison with figures 19 and 20 of Bensley[4] indicates that it is equivalent or superior to that which he secured. In particular the definition of nuclear constituents in figure 2*a* is striking and serves as a confirmation of the results to which Bensley assigns considerable significance. Our fixations of animal material were uniformly good and cytological detail was preserved as faithfully as in the control preparations prepared according to conventional techniques.

By contrast, our preliminary attempts to apply the freezing-drying procedure to plant tissue have not been so uniformly satisfactory. Anthers of *Lilium longiflorum* var. *grandiflorum* from archesporial stages to mature pollen and root tips of *Allium cepa* were treated according to the above detailed procedure, controls being

DESCRIPTION OF FIGURE 2

Photomicrographs of cells treated by the freezing-drying method. (*a*) Rat liver cells, dehydrated 14 days at −32°C. and infiltrated with paraffin oil; × 1900. (*b*) Mitochondria in early prophase pollen mother cells of *Lilium*, dehydrated 14 days at −32°C. and infiltrated with paraffin; × 540. (*c*) Polar view of first meiotic metaphase of pollen mother cell of *Lilium*, dehydrated 7 days at −22°C. and infiltrated with xylol; × 810.

fixed in a modified Navashin's fluid in every case. The anthers were split longitudinally and, in some cases, transversely also. Various periods of dehydration at −22°C. and −32°C. were investigated. The first evidence of a distinction in reaction to the freezing-drying technique between the animal and plant tissues employed was the difficulty in infiltration of the latter. Thus, the rat tissue was thoroughly infiltrated in one or two minutes while from 30 to 120 minutes were required to obtain a similar condition in lily anthers. As judged by the appearance of the control and the evidence of others as to the cytology of the classical material which we employed, the fixation was not as uniformly exact as in the case of the rat tissues correspondingly treated. Thus, a certain amount of apparent shrinkage was characteristic although particular cell layers showed little plasmolysis and, indeed, the volume of the protoplast usually did not appear to be greatly diminished but the stretching or swelling of the tissue in general causes the cells to separate and thus simulate shrinkage (cf. Fig. 2*b*). Again, the tendency of the protoplasm to appear striated (cf. Fig. 2) or markedly vesicular aroused doubts as to the perfection of the fixation.

The slower rate of dehydration to be expected for plant as contrasted with animal tissue and, in particular, the range of distinction in water content and concentration of mineral salts in the sequence of tissue systems present in, for example, a cross-section of an immature anther may make uniformly good fixation of all cells of such an organ as difficult to obtain with the freezing-drying method as it is with conventional procedures for preparing plant tissue for microscopical examination. On the other hand, we have not as yet canvassed the range of combinations of temperature, pressure and time of treatment, the theoretical background of which has been commented upon above. The temperature relation has to a certain extent been determined in that it appears that dehydration for two weeks at a brine temperature of −32°C. is more satisfactory than at −22°C. (cf. Scott[2]), even though the vapor pressure is three times as great at the higher temperature. From our experience and that of others, the period necessary for dehydration of frozen tissues is as yet largely an arbitrary matter and sufficient attention has not been given to standardizing the volume of tissue to be treated.

It is clear that, if we are not obtaining by this technique a new picture of the structure of protoplasm in the actively living plant cell, a rather striking view of the consistent reaction of the living substance to unique experimental conditions is here available. Whatever may be the degree of artefact induced by the type of freezing-drying procedure so far employed, we are convinced that certain significant cytological phenomena are more readily or better demonstrated by this technique than those hitherto employed. In figure 2*b* the character and distribution of mitochondria in early prophase pollen mother cells of *Lilium* are strikingly

shown. As already noted, the striated condition of the cytoplasm and the general tendency to cell separation rather than plasmolysis is to be seen in this photomicrograph. The character of the cytoplasm in the area occupied by the early metaphase achromatic figure and the stages of early cytokinesis give somewhat unfamiliar cytological pictures. Of more importance is the reaction of nuclear constituents. Figure 2*c* shows a polar view of first meiotic metaphase in a pollen mother cell of the lily in which, even at this relatively low magnification, the chromonematic structure of the bivalents is clearly evident. The clearness and sharpness with which it is possible to demonstrate details of chromosome structure in mitosis and meiosis in preparations of lily anthers by the freezing-drying technique has not been equaled by other methods and a more detailed description of the evidence obtained and its application to current cytological and genetic postulates is in preparation.

In general, our results indicate that for study of finer details of nuclear and cytoplasmic structure in plant cells the Altmann freezing-drying technique deserves thorough investigation and it appears possible that with it a most precise and uniform fixation of most plant tissues can be obtained.

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[1] Policard, A., *Protoplasma*, **7**, 464 (1929).

[2] Scott, G. H., *Ibid.*, **20**, 133 (1933).

[3] Altmann, R., *Die Elementarorganismen*, Leipzig, Veit, p. 27 (1894).

[4] Bensley, R. R., *Anat. Rec.*, **58**, 1 (1933).

[5] Bensley, R. R., and Gersh, I., *Ibid.*, **57**, 205, 217, 369 (1933).

[6] Gersh, I., *Ibid.*, **53**, 309 (1932).

[7] Alty, T., *Proc. Roy. Soc.*, **A131**, 554 (1931).

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*REVERSIBLE COAGULATION IN LIVING TISSUE. XII**

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In an earlier paper[1] it was shown that sodium rhodanate counteracted the effect of sodium amytal on rabbits. At that time we had not worked out the theory of sleep and we consequently did not know that certain doses of sodium rhodanate would increase the time of anesthesia, while other doses would shorten it. This oversight was corrected in the paper[2] on "The Agglomeration Theory of Sleep."

The fact that different doses of sodium rhodanate may produce what are apparently diametrically opposite results has been of great help to

the physiologists and medical men who disagreed with us. By concentrating, presumably unconsciously, on the data which disagreed with what they believed to be our view, they were able to satisfy themselves that we were wrong and they said so with much enthusiasm. It was very foolish, because the antagonism between sodium rhodanate as a peptizing agent and such agglomerating agents as morphine, alcohol and sodium amytal has been proved for man and the use of ephedrine to counteract the action of sodium amytal on man is regular medical practice. It seemed to be desirable, however, to obtain additional experiments to show that what holds for man also holds for rabbits.

In all of the experiments described in this paper the sodium amytal was given by intravenous injection, generally into the marginal vein of the ear. The first dose of sodium rhodanate (ten per cent solution) was always given in the same way. Occasionally a second dose was given subcutaneously. Except in the cases of rabbits numbered 18, 19, 20 and 21, a five per cent solution was used of amytal dissolved in distilled water in presence of sodium hydroxide. The solutions were prepared by adding 4 cc. distilled water to one gram amytal, shaking in order to wet the amytal thoroughly and then adding 6 cc. of 0.876 *N* NaOH solution. This was followed in not more than three minutes, during which time the container was shaken, by 10 cc. of distilled water. The solutions of sodium amytal were never more than two hours old when they were used. The sodium amytal solution that was used for rabbits numbered 23–29, inclusive, was prepared by adding 8 cc. distilled water to one gram amytal, shaking to wet the amytal thoroughly and then adding 12 cc. of 0.876 NaOH solution. The solution used for rabbits numbered 20 and 21 was a ten per cent solution of sodium amytal. The amytal for rabbits numbered 18 and 19 was dissolved in anhydrous C. P. glycerol to make a four per cent solution.

The time of anesthesia for rabbits with sodium amytal varies very much, nearly eight to one, from rabbit to rabbit and with the same rabbit at different times. Similar results with the barbiturates in general have been recorded by Sollmann[3] and by Stevenson and Milks.[4] Even the order and degree of the responses to stimulation of the lid, corneal and whisker reflexes may vary from rabbit to rabbit and are therefore worthless for comparison. We have, however, found a comparison test that seems to be fairly reproducible for rabbits which have received both sodium amytal and sodium rhodanate. The determination of what we call the crawling to normal hopping time gives results which are independent within limits of the amount of amytal given and of the breed and weight of the rabbits.

There are two stages, symptomatically, to amytal anesthesia as considered here. The first stage is that of a variable degree of unconscious-

ness. It is considered that this stage ends when the rabbit makes movements akin to clonic movements in an attempt to crawl, or actually crawls. The second stage is that during which the rabbit progresses from uncoördinated attempts to crawl, through periods of actual crawling, ataxic hopping, to normal hopping. As the rabbits go through the second stage of anesthesia, any impartial observer can see the differences between rabbits that have and have not been given sodium rhodanate. Rabbits given sodium amytal alone are unconscious for a period of time; they then begin to make weak voluntary movements which increase in vigor until they begin to make more or less weak and uncoördinated attempts to crawl. This merges into a period during which the rabbits do crawl in an ataxic fashion. The ataxic crawling is followed by a longer period of time during which the rabbits hop about in an ataxic manner. Finally, they hop normally. Frequently, these rabbits made attempts to crawl, or actually did crawl two to three feet, following which they lay stretched out on their sides apparently unconscious, for up to five minutes, after which they resumed their attempts to crawl. Immediately prior to their attempts to crawl, these rabbits showed continuously increasing voluntary movements, so that it was possible to tell that they would soon begin trying to crawl. Due to the lack of coördination the attempts to crawl did not resemble clonic movements at all. The muscular action of the hind legs was weak. As soon as the rabbits began ataxic hopping, they remained in motion as much as a normal rabbit would until the time when they began normal hopping, i.e., without ataxia.

The recovery picture for the rabbits given sodium rhodanate is quite different and varies periodically with the dose of sodium rhodanate. Rabbits numbered 1, 13, 14, 17, 18, 23, 25 and 26 made the sharpest recovery from the anesthetic. With these rabbits there was no period of sleep during the time from crawling to normal hopping. In contrast to the rabbits that were given only sodium amytal, all of the rabbits that were also given sodium rhodanate made more concerted efforts to crawl, at least until they reached a normal sitting position. The hind-leg action of the rabbits that were also given sodium rhodanate was distinctly stronger when they first began to crawl—in several cases almost of normal strength—than of those that were given only sodium amytal. The first hind-leg movements of the rabbits that were given sodium rhodanate were more coördinated than those of the controls; in several cases they resembled clonic movements, due to the greater degree of coördination. In the cases of the rabbits numbered 14, 17, 23, 25, 26, and to a lesser extent 18, they initiated very strong and sustained efforts to crawl without going through a stage of continuously increasing voluntary movements immediately prior to the attempts to crawl, in contradistinction to the controls. Rabbits numbered 23, 25 and 26 (which were litter mates with

27, 28 and 29) began ataxic hopping without even going through the stage of crawling or of attempting to crawl. This behavior was never observed with the controls. It is interesting to note that rabbit number 25 ate "rabbitine" before it was able either to crawl or to hop; while rabbit number 26 ate it within three minutes after it began to hop. These types of results were obtained with doses of sodium rhodanate from 49 mg./kg. up to 136 mg./kg., and again from 176 mg./kg. up to 290 mg./kg., inclusive.

TABLE 1

RABBIT NUMBER	WEIGHT IN KG.	MG./KG. SODIUM AMYTAL	TOTAL TIME OF ANESTHESIA IN MINUTES	TIME FROM CRAWLING TO NORMAL HOPPING IN MINUTES	MG./KG. NaSCN	SEX
1	1.587	60	580	30	290	
1	1.540	60	447	168	0	
2	2.041	60	597	60	147	
2	2.041	60	> 660	...	0	
3	2.268	60	350	45	0	
3	2.268	60	247	30	176	
4	2.268	60	656	135	0	
8	2.921	60	412	50	0	F
8	2.494	60	574	90	159	F
9	2.324	60	336	55	0	F
10	3.089	60	336	50	145	M
11	2.182	50	331	62	0	F
12	2.238	50	265	60	0	M
13	2.580	50	241	77	153	M
14	1.984	50	230	29	136	F
15	1.814	50	132	22	0	F
16	2.834	50	255	66	0	M
17	3.175	50	154	17	110	M
18	1.630	37.5	175	15	116	
19	2.950	37.5	192	35	0	
20	2.050	50	186	51	0	
21	2.200	55	177	51	0	
23	1.076	50	181	12	49	
25	1.247	50	183	6	124	
26	1.302	50	190	11	130	
27	1.267	50	235	33	142	
28	1.470	50	337	98	156	
29	2.580	50	280	64	170	F

Doses of sodium rhodanate between 136 mg./kg. and 176 mg./kg. gave rise to quite a different phenomenon during the stage from crawling to normal hopping. Rabbits given these doses were stronger than the controls when they first began to crawl. From the time that they first attempted to crawl until they were able to hop in a very ataxic manner, there were more sustained general muscular movements of the animals than was the case with the controls. The recovery picture then became

very different from that of the controls or of the rabbits that were given larger or smaller doses of sodium rhodanate. As soon as these rabbits, numbered 2, 8, 10, 13, 27, 28 and 29, began to hop, they would hop a few steps, sleep from five to fifteen minutes in an upright position, hop some more, sleep again and repeat the cycle several times until they were finally able to hop normally. Even when the ataxic hopping had disappeared, these rabbits had a tendency to sleep for longer or shorter

TABLE 2

RABBIT NUMBER	CC./SEC. RATE OF INJECTION OF AMYTAL	BREED	MINIMUM RATE OF RESPIRATION	CHANGE IN RESP. RATE AFTER NaSCN	NaSCN INJECTED AFTER—MIN.
1	0.0345	N. Z. White	12	0	244
1	0.0240	N. Z. White	22	..	...
2	0.0218	N. Z. White	22	−10	224
2	0.0218	N. Z. White	18	..	...
3	0.0245	N. Z. White	28	..	...
3	0.0247	N. Z. White	25	+ 6	175
4	0.0260	N. Z. White	26	..	...
8	0.0218	N. Z. White	30	..	...
8	0.0230	N. Z. White	16	− 1	301
9	0.0207	N. Z. White	18	..	...
10	0.0260	N. Z. White	28	+ 2	231
11	0.0260	Chinchilla	26	..	...
12	0.0300	Chinchilla	28	..	...
13	0.0325	Chinchilla	28	−36	84
14	0.0280	N. Z. White	30	−10	96
15	0.0290	N. Z. White	46	..	...
16	0.0390	N. Z. White	28	..	...
17	0.0250	N. Z. White	30	+ 8	50
18	....	N. Z. White	24	+12	30
19	....	Brown	26	..	...
20	0.0220	Brown	8	..	...
21	0.0266	Brown	22	..	...
23	0.0225	N. Z. White	34	+ 6	85
25	0.0250	N. Z. White	24	+ 7	85
26	0.0276	N. Z. White	40	+ 8	85
27	0.0263	N. Z. White	30	+ 4	83
28	0.0326	N. Z. White	26	−11	85
29	0.0328	N. Z. White	..	+14	88

periods. Whenever any control rabbit exhibited periods of what may be called sleep it was observed only in the interval from the time that the animal attempted to crawl until it did actually crawl around a great deal. In other words they did not sleep in the upright rabbit position, as did the rabbits with doses of sodium rhodanate between 136 mg./kg. and 170 mg./kg., inclusive. The rhodanate rabbits that went through a period of what appeared to be approximately normal sleep gave the impression that, if they had hopped around as much as the other rabbits

treated with sodium rhodanate, the time from crawling to normal hopping would have been considerably shorter. If one plots the time from crawling to natural hopping against the amounts of sodium rhodanate injected, one gets curves which can apparently be duplicated with an unexpected degree of accuracy. Some of the general data are given in tables 1 and 2, and the important ones are plotted in figure 1. The curves are based on

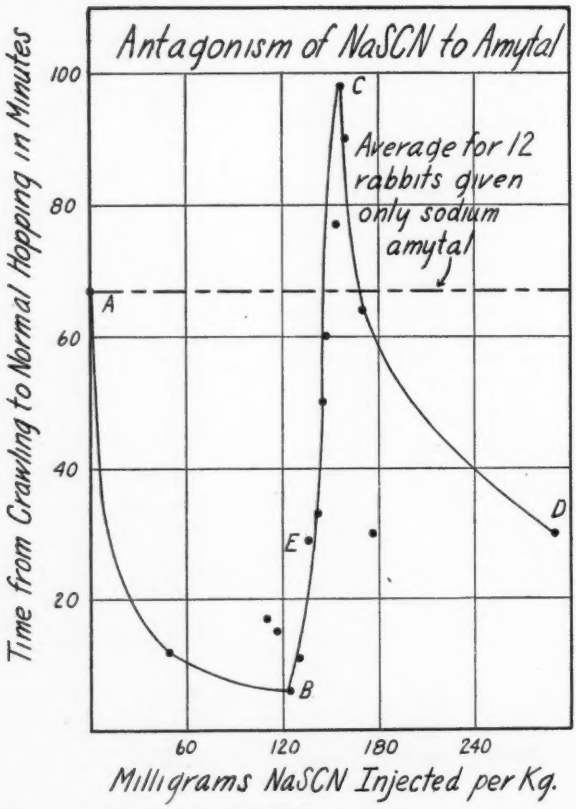

FIGURE 1

two sets of data obtained four months apart. They coincide unexpectedly well.

The injection of small amounts of sodium rhodanate decreases the time of recovery as measured, along *AB*. Along *BEC* injection of sodium rhodanate decreases the time of recovery. At *C* the time of recovery is nearly fifty per cent greater than at *A*. Along *CD* injection of sodium

rhodanate cuts down the time of recovery. It is easy to see that an enthusiastic physiologist, who did not work systematically and who obtained the highest six values on *BCD*, would believe that the effect of sodium rhodanate is absolutely haphazard.

Along *ABE* the rabbits recovered sharply without periods of sleep between the time that they began to hop and the time that they were able to hop normally. From *E* to *C* there were periods of sleep in the interval from the first hopping to normal hopping. From the point *C* to the point *D* the rabbits exhibited a lessening tendency to sleep during the period from ataxic to normal hopping.

Our present explanation of the curves is that small amounts of sodium rhodanate force the sodium amytal off the sensory nerves, increasing their irritability and the consequent tendency to wake the sleeper. Some of the amytal displaced from the sensory nerves is undoubtedly forced on the proteins of the centers of consciousness, thereby increasing the tendency to sleep. When more sodium rhodanate is given, it brings the sensory nerves more nearly to normal, thus permitting sleep. Any forcing of the amytal on the centers of consciousness will increase this effect. Along *CE* we believe that the sodium rhodanate is displacing the sodium amytal from the proteins constituting the centers of consciousness.

Invariably, when the anesthetized rabbits were given sodium rhodanate, they struggled quite violently during the injection and either tried to rise or made a few attempts to crawl, or did both. They then lapsed back into the narcosis and, in some cases, slept for several hours afterward. This is due to the presence of a wave of sodium rhodanate of higher concentration than the final equilibrium concentration in the blood-stream. This is because the sodium rhodanate does not reach its equilibrium value in the blood instantaneously on account of the presence of the adsorbed amytal.

Of fifteen rabbits treated with sodium amytal and sodium rhodanate, only one, numbered six, which is not in the tables of data, exhibited toxic effects after the end of the anesthesia. It was given 60 mg./kg. of sodium amytal and 150 mg./kg. of sodium rhodanate. This rabbit developed a hind-leg paralysis before recovering from the anesthesia; but nevertheless it attempted to rise after the injection of the sodium rhodanate. We suspect that perhaps the sodium rhodanate was injected too rapidly. No peculiar inability to control the hind legs different from that of the controls was observed in any other case. Furthermore, one rabbit, numbered four, that received only sodium amytal, developed a severe paralysis of the hind legs about one week after the experiment. Any report mentioning a number of paralyses of the hind legs is therefore based on poor experimental technique, on the use of impure sodium thiocyanate, on the use of potassium thiocyanate or on some combination of these.

When sodium rhodanate is injected intravenously and is followed by sodium amytal, the sodium rhodanate, which is adsorbed strongly by the serum proteins, will retard adsorption of the sodium amytal at least to some extent. The result of that will be a higher free concentration in the blood-stream than would otherwise be the case. This should result in greater toxic action of the sodium amytal for those organs that are not protected by the peptizing agent, unless the peptizing agent can be got into the body in high enough non-toxic doses to protect all of the organs that can be affected by sodium amytal. Two cases of increased toxic action of sodium amytal have been observed. One rabbit was given 272 mg./kg. of sodium rhodanate intravenously. Thirty-five minutes later it was given 60 mg./kg. of sodium amytal, using a five per cent solution and injecting it at the rate of 0.024 cc. per second. As soon as the sodium amytal solution began to go in, the animal struggled violently, keeping this up for forty seconds. The excitation was more violent and sustained than with the rabbits that were given sodium amytal first. After forty seconds the animal quieted down. It died within five minutes after anesthesia was induced. The excessive excitation was due to the protecting effect of the sodium rhodanate on the sensory nerves, which were not carried through the irritable stage so quickly as usual. When 280 mg./kg. were given and the sodium amytal solution was administered somewhat more rapidly, similar phenomena were observed. The rabbit struggled more violently but for only twenty seconds. It was dead when the injection was completed.

We have confirmed our own previous results; we have confirmed the results of those who differ from us, results which we had ourselves predicted; we can get either set of results at will. Experiments purporting to show no antagonism between sodium rhodanate and such agglomerating agents as anesthetics, narcotics, etc., will be ignored in the future. They are due either to faulty technique, to faulty interpretation or to some combination of the two.

Professor Herbert L. Davis called our attention last winter to some lectures by Dr. Wolfgang Pauli, translated into English by Martin H. Fischer and published in 1906. Pauli used sodium thiocyanate for some physiological disturbances because sodium is the least harmful cation and because thiocyanate ion is the most effective in peptizing proteins.

"The existence of a sedative action was tested on a series of neuroses and organic nervous diseases in which the signs of an increased excitability, sleeplessness, increased reflexes, tremors, etc., existed. Ten cases in all were studied: two cardiac neuroses, with signs of general neurasthenia; three neurasthenics; two general paretics; one tabes dorsalis with increased irritability; and two climacteric neuroses. Within two to five days the patients became much quieter, and with further use of the drug

a very decided improvement in even the most disturbing symptoms took place. The fear, restless sleep, headache and dizziness became less, the troublesome congestions of the climacteric women passed away, and a sense of rest repeatedly took the place of weariness in the patient. . . .

"My last observations are taken from a group of patients with syphilitic headaches—two men and two women. In the men there was a history of syphilitic infection; in the women abortions and still-births had occurred. One man and one woman had a year previously suffered from the same symptoms, which had disappeared promptly after the use of iodides, and only after these. The headaches were in all cases very severe, and in three of them typically nocturnal in character, particularly during the weeks when this symptom first developed. Sensitiveness of the skull upon percussion also existed.

"The syphilitic character of the pains was therefore established definitely in these cases, so far as this is clinically possible. The diagnosis was further strengthened by the uselessness clinically of physical heating methods and antineuralgic methods.

"The effect of the sulphocyanate in these cases was so prompt and so clearly beneficent, even in the first few days after administration, that its medicinal properties cannot be doubted. The effect upon the patients proved in all cases to be a lasting one. The two patients, who had for similar symptoms previously undergone a treatment with iodides, agreed that the feeling of relief had this time come much more promptly. In one of the patients, who could tolerate drugs only badly and who had a year previously suffered from severe iodism, the sulphocyanate was administered in two small enemas daily."

Pauli did not use sodium rhodanate for morphinism, alcoholism, insomnia or psychoses, and he seems never to have seen the problem as a whole or to have considered the effect of agglomerating agents; but he may be said to have confirmed our conclusions twenty-five years before we thought of drawing them. After Claude Bernard, Pauli is the pioneer in this line of work. It would have made a great difference to the world if Pauli had appreciated the full bearing of his work and had gone on with it, instead of shifting to other applications of colloid chemistry.

* This work is done under the programme now being carried out at Cornell University and supported in part by a Grant from the Heckscher Foundation for the Advancement of Research established by August Heckscher at Cornell University.

[1] Bancroft and Rutzler, *Proc. Nat. Acad. Sci.*, **17**, 105 (1931).

[2] Bancroft and Rutzler, *Ibid.*, **19**, 74 (1933).

[3] Sollmann, *A Manual of Pharmacology*, 770 (1932).

[4] Stevenson and Milks, *Cornell Veterinarian*, **21**, 161 (1931).

[5] Pauli, *Physical Chemistry in the Service of Medicine* (translated by Martin H. Fischer), 81, 87 (1907).

GENETIC EVOLUTIONARY PROCESSES

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Communicated August 11, 1934

To the geneticist, at least, and presumably to most biologists, organic evolution implies preliminary changes of some sort in hereditary potentialities. The rôle played by the environment, either external or internal to the organism, in the causation of these hereditary or genetic changes, involves what are presumably the most basic of all evolutionary processes. If this be granted, it follows that the genetic changes, once they have been induced, must operate sometimes as the first steps in the origin of species. The subsequent rôle of the environment in determining survival values may be looked upon as secondary.

There have been recent noteworthy achievements in the induction of genetic changes by various experimental methods. As is well known, it has been proved that diverse genetic changes can be caused by irradiation, by extreme temperatures and by treatment with certain chemical substances. But the relative importance of these or other causal agencies in organic evolution remains for the future to disclose.

Meanwhile it has been possible to learn something about the various types of genetic change which have played a rôle in the evolution of groups of organisms by the study of the organisms themselves. The method used includes four steps: (1) determination of relationships within and between natural groups of species by means of such criteria as comparative morphology, geographic distribution, chromosome number and morphology, and cytogenetics; (2) comparison of the evidence on classification so derived; (3) the synthetic treatment of this evidence in respect to the phyletic relations of the species under consideration; (4) derivation of conclusions regarding the nature of the genetic changes involved in the evolution of a given group of species.

For some twenty years the writer has been engaged in such a study of a large, cosmopolitan group of plants in the Compositæ. This largest of all the families is generally regarded as the most recently evolved division of dicotyledonous plants. The group under investigation is the genus *Crepis* which includes about 225 species. Nearly half of these species, representing all subdivisions of the genus, have been collected in living condition and subjected to intensive investigation. Recently some attention has also been given to certain species representing several closely related genera. Detailed reports of this research are published elsewhere.[1,2,3] It is the purpose of the present paper to summarize the conclusions reached regarding the processes of genetic change which have

made possible the development of the genus *Crepis*, to state a hypothesis as to the genetic processes involved in the evolution of the whole subtribe, Crepidinæ, and to point out the general significance of these results.

I. *Genetic Processes Involved in the Evolution of Crepis Stated in the Order of Relative Importance.*—(1) In view of the evidence that there is one most primitive chromosome number and type of genom in *Crepis* and that all other chromosome numbers and types of genom were derived therefrom, it follows that the primary evolutionary process which has made possible the development of the genus, as we now know it, is some mode of transformation by which 8-chromosome and 6-chromosome species have been derived from 10-chromosome ancestors. Although the actual steps in this process of chromosome transformation, resulting in the disappearance of a pair of chromosomes *as such*, has not been proved experimentally, yet a mechanism is known which makes such a fundamental genetic change possible. The term generally applied to this mechanism is translocation. Reciprocal translocation between non-homologous chromosomes has been demonstrated cytogenetically in maize.[4] Such interchange of segments between non-homologous chromosomes is known to result occasionally in the elimination of chromosome fragments. Translocations between non-homologous chromosomes without reciprocal interchange have been induced experimentally in Drosophila[5] and shown to be followed sometimes by the formation of fragments which must be eliminated eventually. Such translocations and fragments have been observed cytologically in *Crepis* and other plants; and Navashin[6] has proposed a hypothesis of evolution in chromosome number based on these observations. This hypothesis provides for both increase and decrease in number of chromosome pairs. While it remains to be proved that 8-chromosome species can originate from 10-chromosome ancestors, all the evidence from the study of species relations indicates that they did so originate in *Crepis*.

(2) Next in importance is interspecific hybridization sometimes followed by amphidiploidy, the complete doubling of both specific genoms present in an F_1 hybrid, which has been proved experimentally in a number of instances to result in potential new species; and interspecific hybridization followed by backcrossing on the parental species or by crossing with a third species, either of which events may sometimes produce constant new derivative forms which are potential new species. It has recently been shown by Müntzing[7] that "crossing-over in species hybrids between homologous segments of structurally different chromosomes may give rise to a high number of new chromosome types, some of which are probably of evolutionary value." In *Crepis* there is abundant evidence that interspecific hybridization is second in importance only to chromosomal transformation in the evolution of the genus.

(3) Third comes polyploidy. Tetraploid forms of existing species of *Crepis* are fairly common and a number of distinct species are known which apparently arose through tetraploidy. A few higher grades of polyploidy are also known in *Crepis*.

(4) Superimposed upon and operating concurrently with all the foregoing is gene or point mutation. Many species of *Crepis* are polymorphic, consisting of more or less well-defined geographic races or subspecific entities. Some of these have been shown to differ only with respect to certain genes.[1] It is most logical to infer that they are in process of differentiation as a result of gene mutation and selection under different environmental conditions, the process being accentuated by some sort of isolation.

II. *Hypothesis as to the Basic Processes Involved in the Evolution of the Crepidinæ.*—The subtribe Crepidinæ, of the Chicory Tribe of the Compositæ, includes over twenty genera, several of which are large cosmopolitan groups like *Crepis*. From a study[3] of the geographic distribution of these genera it became evident that the subtribe is a natural group with a common region of origin. In the light of this evidence and on the basis of such information as is at present available concerning chromosome numbers in *Crepis* and these related genera the following hypothesis was proposed. (1) The original stock from which the whole subtribe was derived consisted of one or more 10-chromosome species (see I, 1 above); (2) new species with 8 chromosomes originated from the original 10-chromosome species through some such process as reciprocal translocation between non-homologous chromosomes or non-reciprocal translocation from one non-homologous chromosome to another, leading eventually to the absence of one pair of chromosomes; (3) the genus *Crepis* arose from the complex of 10-chromosome and 8-chromosome species thus created; (4) the other genera in the Crepidinæ originated through interspecific hybridization between 10-chromosome and 8-chromosome species (or between 8-chromosome species) followed by amphidiploidy; (5) speciation and evolution, within *Crepis* at least, have involved the same genetic processes together with autopolyploidy and, concurrently with all these processes, gene mutation has played an important rôle in differentiation.

These propositions are submitted as the most logical conception of the early course of evolution in the Crepidinæ in view of the evidence at present available. Future research on *Crepis* and especially on the genera closest to *Crepis* may result in modification or extension of the hypothesis, but it is believed that the primary genetic processes involved in the evolution of this large group of plants have been discovered. If this working hypothesis shall ultimately become well established by additional evidence, this result will necessitate the recognition of gross chromosomal

alterations as having played a more important rôle in the evolution of certain large groups of higher plants than has gene mutation.

III. *General Significance of the Conclusions Reached About Evolutionary Processes in Crepis and the Hypothesis Concerning Evolution of the Crepidinæ.*—In an earlier publication[8] the writer called attention to the inadequacy of the gene-mutation theory of evolution[9,10] in accounting for differences in chromosome number among related species and for the gross chromosomal differences which characterize various major groups of animals and plants. It was therein suggested that future research may disclose other types of genetic change which have been of primary importance in evolution. This criticism and suggestion have been substantiated by the conclusions and hypothesis summarized above.

Considerable evidence of a similar nature has been accumulated in recent years as a result of other investigations, especially on plants. The importance of interspecific hybridization, followed by amphidiploidy or by backcrossing or crossing with other species, has become increasingly evident; while the rôle of polyploidy in creating arithmetical series of chromosome numbers in certain genera has been emphasized. That such genetic processes must be considered as causes of evolution has been recognized by Haldane,[11] Darlington,[12] Hurst[13] and Sansome and Philp.[14] But there is a tendency still to think of mutation in the strict sense, i.e., of gene mutation, as the only genetic evolutionary process of general importance.

In *Crepis*, however, it has been possible, by the synthetic application of several criteria of relationship, to determine the most basic chromosome number and type of genom in the genus and to infer therefrom the nature of the foremost genetic process which has operated in the evolution of this group of plants. This process of genom transformation by which species with lower chromosome numbers were derived from species having a higher chromosome number, probably without the intervention of interspecific hybridization, was reported in an earlier contribution.[1] But it has not as yet been generally recognized as a cause of evolution.

Whether this transformation of genoms came about by means of reciprocal translocation between non-homologous chromosomes or by some other process is of minor importance compared with the evidence that it must have occurred as the first step in the evolution of a large genus of plants. Of more general significance is the conclusion that several kinds of genetic change are of more importance in the evolution of *Crepis* than is gene mutation. Also in the hypothetical scheme of evolution of the Crepidinæ chromosomal transformation and interspecific hybridization are necessarily the primary genetic processes. While gene mutation has doubtless been operating concurrently with these primary processes, in certain groups of plants at least it must be viewed as a secondary or con-

comitant process. At the same time it is undoubtedly an essential process in subspecific and specific differentiation because it provides the Mendelian differences which are necessary for the operation of natural selection or natural elimination and the consequent establishment of ecotypes, geographic races, subspecies and species having the same type of genom as the species from which they developed.

The mathematical theory of natural selection, as developed by Fisher,[15] Wright[16] and Haldane,[11] is concerned only with selection in Mendelian populations. In this connection the following quotation from Haldane is significant: "If much of the investigation here summarized has only proved the obvious, the obvious is worth proving when this can be done. And if the relative importance of selection and mutation is obvious, it has certainly not always been recognized as such." The present writer[8] has emphasized the necessity, on the basis of common sense unaided by mathematical analysis, of recognizing natural selection as an important evolutionary agency. The heritable variations upon which natural selection is continually operating, however, comprise various other categories besides the Mendelian differences produced by gene mutation. The importance of these genetic evolutionary processes other than gene mutation must be recognized. The hereditary changes they produce fall outside the category of single gene differences. The morphological and physiological effects of these other types of genetic change result from shuffling, recombining, adding and losing whole blocks of genes or whole chromosomes and they frequently produce something new in nature, something which could not have resulted from gene mutation alone. To what extent a mathematical theory of natural selection can be applied to the results of these other categories of genetic change remains for the future to disclose.

The relative importance of chromosomal transformation as compared with gene mutation in the evolution of genera, families and orders of plants and animals also remains to be determined. Some conception of the rôle played by chromosomal transformation may be gained by a systematic review of the present available evidence on the chromosomes of species. Such a review must take into account not only chromosome number but also differences in size and shape of the chromosomes of different species. This will greatly reduce the amount of dependable data. Meanwhile, further intensive research on especially favorable groups, for example, such groups as the genus *Crepis* and several related genera, should yield additional evidence bearing directly on this important question.

[1] Babcock, E. B., and Navashin, M., *Bibliog. Genetica*, **6**, 1–90 (1930).

[2] Babcock, E. B., and Cameron, D. R., *Univ. Calif. Pub. Agr. Sci.*, **6**, in press (1934).

[3] Babcock, E. B., *In Setchell Anniversary Volume, Univ. Calif. Press*, in press (1934).

[4] McClintock, B., *Zeitsch. Zellforsch. mikr. Anat.*, **19**, 191–237, cf. lit. cited (1933).
[5] Painter, T. S., and Muller, H. J., *J. Heredity*, **20**, 287–98 (1929).
[6] Navashin, M., *Zeitsch. ind. Abst. Vererb.*, **63**, 224–31 (1932).
[7] Müntzing, A., *Hereditas*, **19**, 284–302 (1934).
[8] Babcock, E. B., *Am. Nat.*, **52**, 116–27 (1918).
[9] Morgan, T. H., *A Critique of the Theory of Evolution* (1916).
[10] Morgan, T. H., *The Scientific Basis of Evolution* (1932).
[11] Haldane, J. B. S., *The Causes of Evolution* (1932).
[12] Darlington, C. D., *Recent Advances in Cytology* (1932).
[13] Hurst, C. C., *The Mechanism of Creative Evolution* (1932).
[14] Sansome, F. W., and Philp, J., *Recent Advances in Plant Genetics* (1932).
[15] Fisher, R. A., *The Genetical Theory of Natural Selection* (1930).
[16] Wright, S., *Genetics*, **16**, 97–159 (1931).

PREFERENTIAL SEGREGATION OF THE FOURTH CHROMOSOMES IN DROSOPHILA MELANOGASTER

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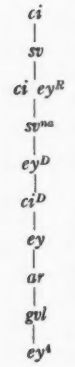
The present study is concerned chiefly with the behavior of a duplicating chromosome derived from the undescribed scute allelomorph known as scute-10-2. This allelomorph, obtained by x-raying scute-10, is associated with a reciprocal 1–4 translocation. The *X*-chromosome is broken near its left end, between the loci of silver and broad, and the short terminal piece (loci from yellow to silver) is exchanged for a section of the fourth chromosome that includes all the known loci of that chromosome (with the possible exception of cubitus-interruptus, which is difficult to test, since this translocation shows the position effect recently described by Dubinin and Sidoroff[1]). The small chromosome, made up of the left end of *X* attached to the spindle-fibre region of 4, may be obtained in individuals (of either sex) that are otherwise normal diploids.[2] If the normal *X*'s of such hyperploids carry the recessive gene for yellow, the flies are wild-type in appearance because of the presence of $+^{y}$ in the duplicating chromosome. This fact has been used, in the present experiments, to study the distribution of the fragment at meiosis.

If hyperploid females of the[3] constitution $\frac{y}{y}$ dupl. $\frac{ey^{D}}{gvl}$ are mated to yellow males there results, not the expected ratio of 1 eyeless : 1 wild-type : 1 yellow eyeless : 1 yellow, but a ratio of 2:1:1:2 (actual numbers obtained, 604 : 307 : 334 : 701). That is to say, the duplicating chromo-

some goes to the same pole as dominant eyeless twice as often as it goes to the same pole as grooveless. This result has been obtained consistently in many different experiments; it is immaterial whether the duplication was received from the same parent as ey^D (observed numbers 347 : 193 : 202 : 397) or from the same parent as *gvl* (257 : 114 : 132 : 304), though in the latter case the appearance is such as to suggest 67 per cent of crossing-over. Similar results may be obtained for other combinations of fourth-chromosome genes, though the disproportion is not always so great (see table below).



There is in fact a definite seriation of the tested fourth chromosomes, such that *ci* is always "preferred" to any other type, while any other type is preferred to ey^4. This seriation is shown in the diagram. It is useful in that it enables one to make accurate qualitative predictions, as has been demonstrated repeatedly.

Quantitatively the results have not yet been worked into a complete scheme, though regularities are obviously present. As the table shows, "preference" is in general strong in combinations of chromosomes that are far apart on the diagram (though never going beyond the 2:1 ratio), weaker in combinations of neighboring chromosomes.

TABLE 1

PERCENTAGE OF ASSOCIATION WITH SCUTE-10-2 DUPLICATION[4]

OPPOSED CHROMOSOME	PREFERRED CHROMOSOME								
	ci	*sv*	*ci ey^R*	*sv^na*	*ey^D*	*ci^D*	*ey*	*ar*	*gvl*
sv	52.2 ±2.5								
ci ey^R	51.0 ±1.1	51.3 ±1.3							
sv^na	53.1 ±1.1	..	56.0 ±0.7						
ey^D	..	..	..	56.2 ±2.2					
ci^D	..	56.1 ±2.4	..	58.1 ±1.8	59.6 ±2.3				
ey	..	62.3 ±2.6	..	..	..	60.3 ±2.7			
ar	..	..	..	56.2 ±3.3	62.6 ±2.2	61.0 ±2.4	..		
gvl	60.7 ±2.6	65.2 ±1.7	67.6 ±1.0	57.8 ±1.2	67.1 ±0.8	55.3 ±1.5	54.6 ±2.0	50.8 ±1.4	
ey^4	..	66.7 ±1.9	..	61.3 ±3.5	..	..	..	59.4 ±4.1	51.8 ±2.0

The sequence appears to be a linear one; but at both ends of the diagram we are concerned with differences that are so slight that more data will be required before they can be considered certainly significant. The

three top chromosomes may be actually equivalent, and the seriation among the last four may require modification.

Other chromosomes are being studied, including two crossover ones. These should throw light on the mechanism concerned, but are not yet ready to report on.

An unexpected result is that these preferences are very much reduced in the male, though apparently still present. In the two best-studied cases, *ci ey*^{R} was preferred to *gvl*, the value being 52.9 ± 0.8; *ey*^{D} to *gvl* with a value of 51.9 ± 1.3. This is not an effect of the *Y*-chromosome, since females with attached *X*'s and a *Y* give the same results as those with separate *X*'s and no *Y*.

A few similar duplicating chromosomes have been tested, in which the spindle-fibre attachment was derived from an *X;* these, though they contained approximately the same "active" region as the scute-10-2 duplication, showed random segregation with respect to the fourth chromosomes tested.

Preliminary experiments with triplo-4 females (not carrying any duplicating fragment) indicate that here also certain combinations give nonrandom segregation. The diagram given above seems to apply, in that chromosomes near the top tend to go to the diplo-4 pole, those near the bottom to the haplo-4 pole. One example may be given. Several different chromosomes were tested against *ey*^{D} and *ci*^{D} at the same time. From such females the percentage of the haplo-4 eggs that contained only the third member of the set was determined, as follows: *sv*, 43.5 ± 2.3; *ci ey*^{R}, 33.8 ± 2.0; *sv*^{na}, 36.4 ± 1.5; *gvl*, 50.7 ± 1.8; *ey*^{4}, 64.2 ± 1.9 (random segregation would, of course, give a value of 33.3).

It does not seem probable that the mutant genes concerned are themselves responsible for the relations described. Tests of crossovers should settle this question; but present indications are rather that the genes merely serve as convenient markers for chromosomes that differ in other respects (perhaps themselves genic). One reason for suspecting this is that two wild-type chromosomes, not yet fully analyzed, lie at different levels (one above *sv*^{na}, the other below *ey*^{D}).

The first hypothesis was that the fourth chromosomes concerned differed in having inversions or other structural differences. Cytological studies by Bridges (in press) show that the *ey*^{D} chromosome does in fact have a gross structural aberration; but nothing unusual has been detected in the other chromosomes, and it seems altogether improbable that structural differences are sufficiently widespread to account for the results.

The "preference" ratio for the duplication appears to have 2:1 as a definite limiting value. Such a simple ratio suggests a simple mechanism for the relation. If the result is referred back to what happens at the conjugation of the chromosomes it becomes possible to picture such

mechanisms; but their elaboration and discussion may be deferred until more information is available.

[1] *Amer. Nat.*, **68**, 381 (1934).

[2] This duplication is regularly eliminated somatically in certain areas. It has been discussed in this connection by Ephrussi (these PROCEEDINGS, **20**, 420 (1934)).

[3] The fourth chromosome genes referred to in this paper by symbols are: *ar*, abdomen-rotatum; *ci*, cubitus-interruptus; ci^D, dominant cubitus-interruptus; *ey*, ey^4, ey^R, recessive eyeless allelomorphs; ey^D, dominant eyeless; *gvl*, grooveless; *sv*, shaven; sv^{na}, naked.

[4] The values listed here as "probable errors" represent the probable percentage deviations from a 1:1 ratio $\left(67.45\sqrt{\frac{1}{4n}}\right)$.

ON THE OCCURRENCE OF AN OREODONT SKELETON IN THE SESPE OF SOUTH MOUNTAIN, CALIFORNIA

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In a previous paper[1] I have noted the occurrence of two distinct types of oreodonts, namely, a leptauchenid and a species tentatively referred to the genus *Promerycochœrus*, in the Sespe deposits as exposed on the flank of South Mountain, Ventura County, California. A third type of oreodont, the genus *Eporeodon*, is now recorded from this upper division of these continental beds.

Location of the Sespe deposits of South Mountain, with reference to the important horizons of the Sespe of the Simi Valley and Las Posas Hills regions, discussed in previous papers published in the PROCEEDINGS, is shown in figure 1. I am privileged to reproduce, through courtesy of the geological staff, Shell Company of California, the columnar section, figure 2, giving the Tertiary stratigraphic sequence at South Mountain. As will be noted in this section, the thick series of Sespe deposits grades upward without stratigraphic break into the marine Vaqueros (Lower Miocene).

The leptauchenids occur lower in the section than the level where *Promerycochœrus? hesperus* was found, and range through a stratigraphic thickness of approximately 1000 feet. The position of *Eporeodon* is shown at Locality 157 Calif. Inst. Tech. Vert. Pale., and lies within the vertical range of the leptauchenids. The occurrence is approximately 1500 feet lower stratigraphically than that of *P.? hesperus*. The skeleton was found in a dominantly maroon-colored shale and siltstone which

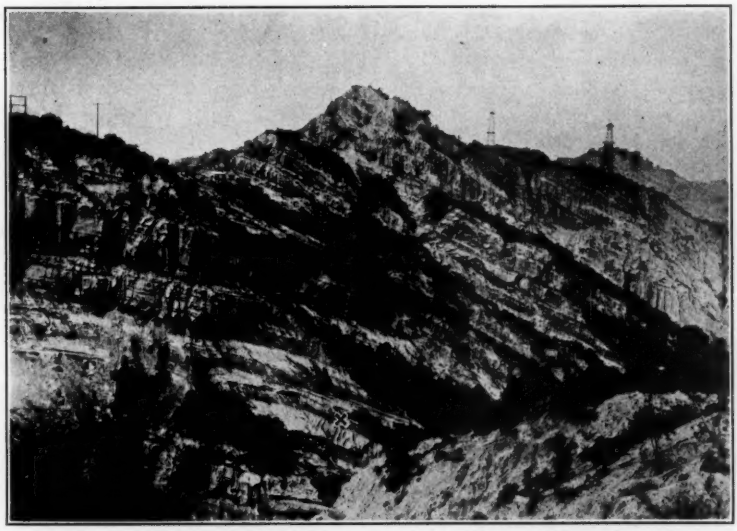

Figure A. View looking northwest across Willard Canyon showing portion of north limb of South Mountain anticline and position of oreodont skeleton at Locality 157 C.I.T. Vert. Pale. (X) in the Sespe deposits. South Mountain, Ventura County, California.

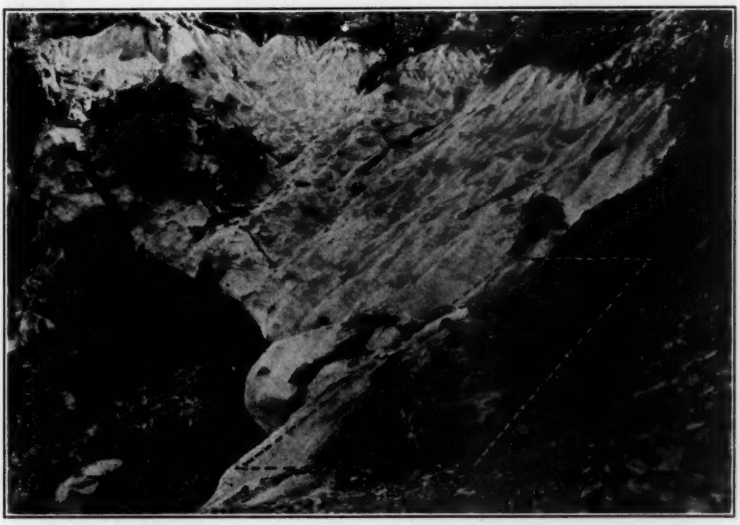

Figure B. Close-up view of Locality 157. Position of maroon-colored shale and siltstone containing oreodont skeleton shown within parallelogram.

PLATE 1

overlay at this locality a stratum of white, cross-bedded sands (see Plate 1, Fig. B). No other vertebrate remains were encountered at this locality.

The specimen (No. 1566 Calif. Inst. Tech. Coll.) had evidently suffered somewhat from exposure prior to burial and is consequently not completely preserved. Plate 2 shows the skeleton in its original position as freed from the rock mass and prepared by H. Anson Wylde. Although the skull lacks the greater portion of the cranium (shown restored in figure), it still retains, fortunately, the tympanic bulla as well as the posterior premolar and molar teeth of the right side. These have been exposed on the reverse side of the plaque.

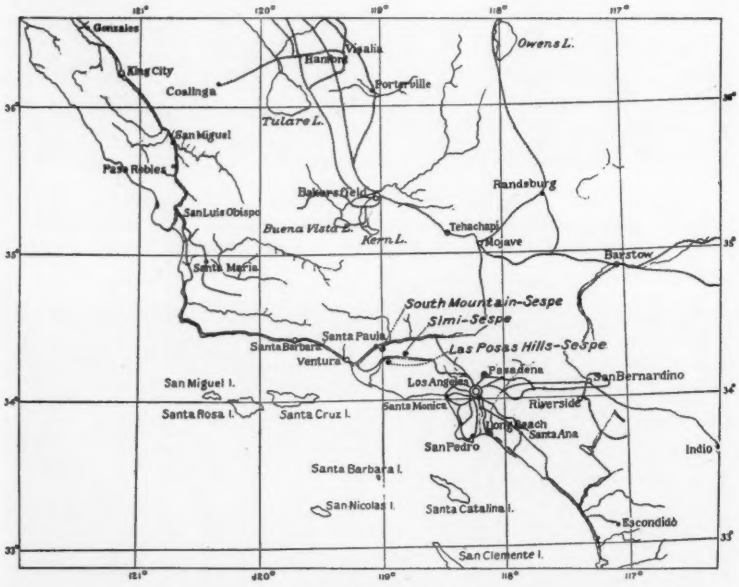

FIGURE 1

Index map showing location of three principal vertebrate fossil occurrences in the Sespe deposits of Ventura County, California.

In presence of the large bulla and in the characters displayed by the premolar teeth, the Sespe form is more like *Eporeodon* than like *Merycoidodon*. The skeleton is of an individual resembling *Eporeodon socialis* in size. In this character it appears to differ from average specimens of *Eporeodon* from the John Day. An antorbital pit was present. The vertebrae of the neck and back are weathered somewhat, but the number of segments comprising the thoracic-lumbar series appears to be 19 or 20.

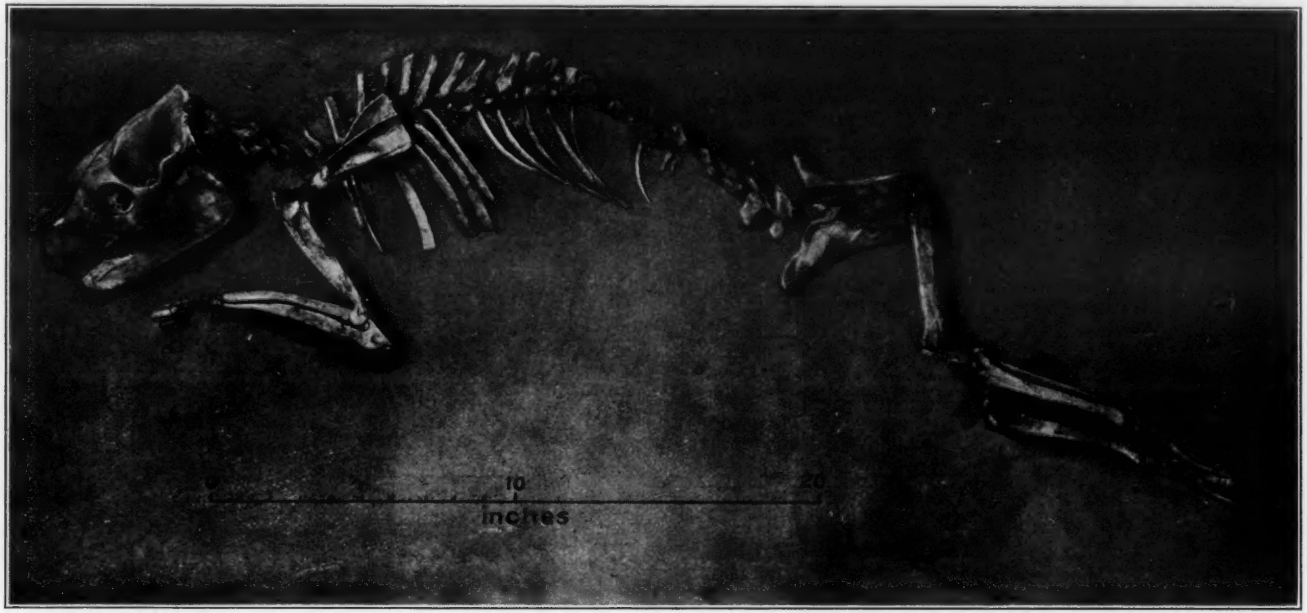

PLATE 2

Eporeodon thurstoni, n. sp.

Skeleton (type specimen, No. 1566 C.I.T. Vert. Pale. Coll.) in original position as freed from rock mass and prepared by H. Anson Wylde. Sespe Upper Oligocene. South Mountain, Ventura County, California.

Thorpe[2] records 13 dorsal and 6 lumbar segments in the vertebral formula for *Eporeodon*. The tail is not preserved in the Sespe specimen. The right pes in No. 1566 shows a rudimentary element in articulation with the navicular and situated above the proximal end of metatarsal II. This may represent a fused entocuneiform and rudimentary metatarsal I. Its postero-distal end is rounded.

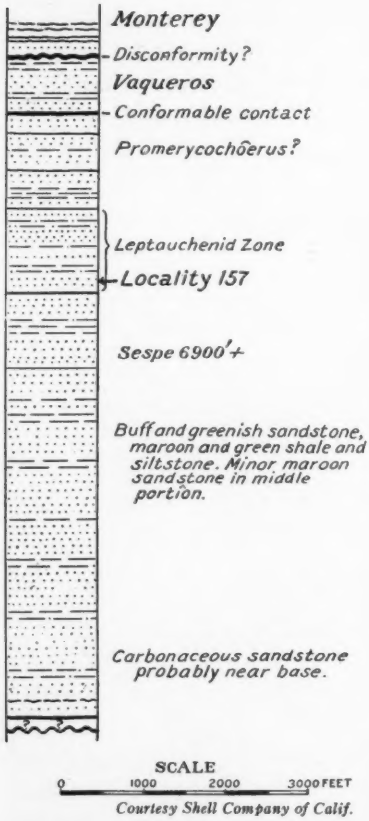

FIGURE 2

Columnar section of Tertiary beds occurring at South Mountain, Ventura County, California. Note position of oreodont skeleton (Locality 157) with reference to occurrences of previously described oreodonts from the Sespe deposits.

In view of the numerous species of *Eporeodon* recorded from the White River and John Day, our type from the Sespe may be found ultimately to represent one of these described forms. It seems desirable, for the present at least, to recognize on the basis of the limited characters noted above the type from the Sespe as a new species, for which the name *Eporeodon thurstoni* n. sp. is here proposed. The species is named for the late James E. Thurston in appreciation of his valuable services in the conduct of the Sespe explorations.

Loomis[3] regards the geologic range of *Eporeodon* as extending from Middle Oligocene to Lower Miocene, the genus occurring in the middle White River, John Day and Lower Rosebud. In western North America, *Eporeodon* is well represented in the middle John Day, and is not absent in the upper John Day. However, *Promerycochœrus* is the more common type to be found in the latter faunal horizon. The leptauchenids remain wholly unrecorded from the John Day.

Evidence afforded by the stratigraphic position and by the morphologic

characters of *E. thurstoni* does not mitigate against the view that the Sespe deposits at Locality 157 on the flank of South Mountain are upper Oligocene in age. It seems probable that the beds included in at least the lower range of the leptauchenids are as old as, or older than, middle John Day. The age relationship between the Sespe at Locality 157 and the John Day may be broadly comparable to that which exists between the Las Posas Hills Sespe (Kew Quarry fauna) and the John Day.

[1] Stock, C., *Carnegie Inst. Wash.*, Publ. **404**, art. 3 (1930).
[2] Thorpe, M. R., *Peabody Mus. Nat. Hist.*, Bull. **2**, 21 (1931).
[3] Loomis, F. B., *Bull. Amer. Mus. Nat. Hist.*, **51**, art. 1 (1924).

ON A THEOREM OF CARLEMAN

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In a paper of Wiener and Paley,[1] the following theorem is proved:

A necessary and sufficient condition that a class of functions C'_A, determined by $\int_{-\infty}^{\infty} f^{(\nu)}(X) \mid^2 dX \leq B^{2\nu}A^2_\nu$, be quasi-analytic is that $\int_0^\infty \frac{dX}{1+X^2} \log \sum_{\nu=0}^{\infty} \frac{X^{2\nu}}{A^2_\nu}$ diverge.

From this theorem, it is possible to prove the fundamental theorem of Carleman[2] concerning quasi-analytic classes over the finite interval. Carleman's theorem states that the functions belonging to C_A, that is, for which $\mid f^{(\nu)}(X) \mid \leq B^\nu A_\nu$ over the interval (0, 1), form a quasi-analytic class if and only if

$$\int_0^\infty \frac{dX}{1+X^2} \log \sum_{\nu=0}^{\infty} \frac{X^{2\nu}}{A^2_\nu} = \infty. \tag{1}$$

To show that divergence is necessary, we proceed as follows. Let $F_0 = 1$, $F_\nu = A_{\nu-1}$. Note that if the integral (1) converges for the sequence A_ν, it does so for the sequence F_ν. From the Wiener-Paley theorem given above, we can construct a function $f(x)$ such that $\int_{-\infty}^{\infty} \mid f^{(\nu)}(X) \mid^2 dX \leq F_\nu^2$ and such that $f(X)$ vanishes with all its derivatives at some point which may be taken as $x = 0$. Moreover, since

$$\mid f^{(\nu)}(X) \mid = \mid \int_0^x f^{(\nu+1)}(X)\, dx \mid \leq [\int_0^1 \mid f^{(\nu+1)}(X) \mid^2 dx]^{1/2} \leq F_{\nu+1} = A_\nu \quad (0 \leq X \leq 1),$$

we have constructed a function belonging to class C_A and vanishing with all its derivatives at $X = 0$. Thus the divergence of the integral is necessary.

We shall now show that the condition is sufficient. Otherwise, a function $f(X)$ exists over $(0, 1)$, vanishing identically over $(0, X)$, not equivalent to zero over $(X_0, 1)$, and belonging to a class C_A for which the integral (1) diverges. Let us now define

$$F(X) = e^{-X} f(1 - e^{-X}), \quad X > 0$$

$$F(X) = 0, \qquad X < 0.$$

We now prove that $F(X)$ belongs to the class C_A' over $(-\infty, \infty)$. For $X > 0$,

$$F(X) = e^{-X} f(1 - e^{-X})$$

$$F^{(1)}(X) = e^{-2X} f^{(1)}(1 - e^{-X}) - e^{X} f(1 - e^{-X})$$

$$\vdots \qquad\qquad \vdots$$

$$F^{(n)}(X) = e^{-(n+1)X} f^{(n)}(1 - e^{-X}) + a_1 e^{-nX} f^{(n-1)}(1 - e^{-X}) + \ldots + a_n e^{-X} f(1 - e^{-X})$$

$$F^{(n+1)}(X) = e^{-(n+2)X} f^{(n+1)}(1 - e^{-X}) + b_1 e^{-(n+1)X} f^{(n)}(1 - e^{-X}) + \ldots + b_{n+1} e^{-X} f(1 - e^{-X}), \text{ etc.}$$

Here $b_1 = -(n + 1) + a_1$, $b_2 = -na_1 + a_2$, $\ldots$, $b_n = -2a_{n-1} + a_n$, $b_{n+1} = -a_n$. If we define $a_0 = a_{n+1} = 0$, we have $|b_\nu| \leq (n + 1)|A_{\nu-1}| + |A_\nu|$, $(\nu = 1, \ldots, n + 1)$.

Now let us assume that $|A_\nu| \leq 2^n n^{2\nu}/\nu!$ Then $|b_\nu| \leq 2^n(2n^{2\nu} + n^{2\nu-1})/\nu! \leq 2^{n+1}(n + 1)^{2\nu}/\nu!$ The inequality is obviously true for the first derivative, and therefore is true for all derivatives. Thus

$$|F^{(n)}(X)| \leq 2^n e^{-X} \left[|f^{(n)}(1 - e^{-X})| + n^2 \left| f^{(n-1)}(1 - e^{-X}) \right| + \frac{n^4}{2!} \left| f^{(n-2)}(1 - e^{-X}) \right| + \ldots + \frac{n^{2n}}{n!} \left| f(1 - e^{-X}) \right| \right], (n = 1, 2, \ldots).$$

Moreover

$$f^{(n-\nu)}(X) = \frac{1}{(\nu - 1)!} \int_0^X f^n(\xi)(X - \xi)^{\nu-1} d\xi, \quad (\nu = 1, 2, \ldots n)$$

Therefore

$$|f^{(n-\nu)}(X)| \leq \frac{1}{(\nu - 1)!} \operatorname{Max} |f^{(n)}(X)| \leq \frac{B^n A_n}{(\nu - 1)!}$$

Thus

$$| F^{(n)}(X) | \leq 2^n e^{-X} B^n A_n \left[1 + n^2 + \frac{n^4}{2!} + \frac{n^6}{2!\,3!} + \ldots + \frac{n^{2n}}{n!\,(n-1)!} \right]$$

$$\leq e^{-X}\, n(2B)^n A_n \left[1 + n^2 + \frac{n^4}{(2!)^2} + \frac{n^6}{(3!)^2} + \ldots + \frac{n^{2n}}{(n!)^2} \right]$$

$$< e^{-X} n(2B)^n A_n \left((1 + n + \frac{n^2}{2!} + \ldots + \frac{n^n}{n!} \right)^2$$

$$< e^{-X} n(2B)^n e^{2n} A_n < e^{-X} (2Be^3)^n A_n.$$

$$\int_{-\infty}^{\infty} | F^{(n)}(X) |^2 dX < C^{2n} A^2{}_n \int_0^{\infty} e^{-2X} dX < C^{2n} A^2{}_n,$$

where $C = 2Be^3$. Thus $F(X)$ belongs to a class C'_A for which the integral (1) diverges, vanishes identically over an interval and is not identically zero. By the Paley-Wiener theorem given above, this is impossible.

[1] "Notes on the Theory and Application of Fourier Transforms," R. E. A. C. Paley and N. Wiener, *Trans. Am. Math. Soc.*, **35**, 348–354. In Chapter I of the 1934 Colloquium Lectures of the American Mathematical Society on "Fourier Transforms in the Complex Domain," by Paley and Wiener, the whole subject, inclusive of the theorem of this paper, will be treated in more detail.

[2] P. 61 of *Les fonctions quasi-analytiques*, by T. Carleman, Paris (1926).

SOME SPECIAL CASES OF THE INDETERMINACY PRINCIPLE

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With the recent increasing development of quantum mechanics a great deal of attention has been paid to the indeterminacy (or uncertainty) principle of Heisenberg. There have been numerous deductions and some applications, notably by Remak[1] who has actually calculated the magnitude of the indeterminacy product for certain special cases, e.g., the harmonic oscillator. In connection with a critical study of the principle undertaken recently by the author other special cases have been studied and it is the purpose of the present paper to report the results. It will first be necessary to introduce some definitions.

If $\bar{q}$ is the mean value of the coördinate, the mean square deviation from the mean is defined as

$$\overline{(\Delta q)^2} = \int (q - \bar{q})^2 |\psi(q)|^2 d\tau, \tag{1}$$

the integration being taken over the whole range of the coördinates. In the above definition $\psi(q)$ is the Schrödinger eigenfunction, the probability amplitude. Similarly for the conjugate momentum

$$\overline{(\Delta p)^2} = \int (p - \bar{p})^2 |\psi(p)|^2 d\sigma, \tag{2}$$

where $d\sigma$ in momentum space corresponds to $d\tau$ in coördinate space. The probability amplitudes are connected by the following relation

$$\psi(p) = \frac{1}{\sqrt{h}} \int e^{\frac{2\pi i p q}{h}} \psi(q)\, dq \tag{3}$$

if for convenience we restrict ourselves to one dimension.

I. *Higher Order Indeterminacies.*—Upon inspection of the various methods of obtaining the indeterminacy relation, it is evident that the actual numerical result is dependent upon the exact method chosen for the derivation, de Broglie finding that the product of the indeterminacies of the position and the conjugate momentum is greater than or equal to h, Planck's constant, while Heisenberg gets $h/4\pi$ for the same product. This suggested as an important problem the calculation of higher order indeterminacies.

We define

$$\overline{(\Delta q)^3} = \int (q - \bar{q})^3 |\psi(q)|^2 dq \tag{4}$$

$$\overline{(\Delta q)^4} = \int (q - \bar{q})^4 |\psi(q)|^2 dq \tag{5}$$

together with similar relations for the momentum. In order to facilitate comparison with the results obtained using the ordinary definition of the indeterminacy, the harmonic oscillator is considered. For this Remak[2] (using the mean square deviation) has already found the indeterminacy relation to be $\sqrt{\overline{(\Delta p_x)^2}\,\overline{(\Delta x)^2}} = h(2n + 1)/4\pi$, where n is a non-negative integer. The results obtained from (3), (4) and (5) are as follows:

$$\sqrt[3]{\overline{(\Delta p_x)^3}\,\overline{(\Delta x)^3}} = 0, \quad \sqrt[4]{\overline{(\Delta p_x)^4}\,\overline{(\Delta x)^4}} = h\sqrt{6n^2 + 6n + 3}/4\pi. \tag{6}$$

The first result is consistent with Remak's which states that the average value of the coördinate is zero, while the value for the fourth root is of the same order of magnitude as that for the square root given above. The difference, however, is another illustration of the fact that the magnitude of the indeterminacy product depends on the definition. It should, of course, be observed that both our fourth order indeterminacy and Remak's

second order one are consistent with the generally established inequality $\Delta p \cdot \Delta q \geqslant h/4\pi$ of Heisenberg.

II. *Linear Oscillator in a Uniform Field.*—A linear oscillator moving in a uniform field affords an interesting if trivial illustration of the indeterminacy relation. The expression for the potential energy is

$$V = {}^1/_2 kx^2 - k\alpha x, \tag{7}$$

where α gives the magnitude of the perturbation, and $k = 4\pi^2 m_0 \nu_0$, m_0 being the mass of the particle, and ν_0 the frequency. The eigenfunctions[3] are

$$\psi_n(x) = e^{-(x-\alpha)^2/2a^2} H_n\{(x - \alpha)/a\}/N_n, \tag{8}$$

where $a^2 = h/4\pi^2 m_0 \nu_0$, N_n is a normalizing factor, and H_n is the Hermite polynomial of order n. After some manipulation, following Remak's scheme in general, it turns out that

$$|\psi(p_x)|^2 = 2\pi a^2 e^{-4\pi^2 a^2 p_x^2/h^2} H_n^2(2\pi a p_x/h)/hN_n^2, \tag{9}$$

and $\sqrt{\overline{(\Delta p_2)^2}\,\overline{(\Delta x)^2}} = h(2n + 1)/4\pi$.

This last result is identical with the result for the harmonic oscillator which is not surprising when one considers that the potential energy may be written in the form

$$V = {}^1/_2 k(x - \alpha)^2 - {}^1/_2 k\alpha^2, \tag{10}$$

which shows that the average value of x is shifted from $x = 0$ to $x = \alpha$, a change which would have no effect on $\overline{(\Delta x)^2}$.

III. *Anharmonic Oscillator.*—The study of the anharmonic oscillator is of interest both because of the possible practical applications of the results obtained and the fact that the eigenfunctions do not appear to be available in the literature.

The potential energy of such an oscillator is[4]

$$V = {}^1/_2 kx^2 + k\alpha x^3. \tag{11}$$

The eigenfunctions are calculated by the Schrödinger method of perturbations. We write

$$E = E_n + \lambda\epsilon_1 + \lambda^2\epsilon_2, \text{ and } \psi = \psi_n + \lambda\varphi_1 + \lambda^2\varphi_2, \tag{12}$$

where ϵ_1 and ϵ_2 are the first and second order correction terms to the energy and φ_1 and φ_2 the corresponding correction terms to the unperturbed eigenfunction. E_n and ψ_n, the eigenenergies and eigenfunctions for the unperturbed case—the harmonic oscillator—are

$$E_n = h\nu_0(n + {}^1/_2); \; \psi_n = e^{-x^2/2a^2} H_n(x/a)/N_n, \tag{13}$$

the meaning of the various symbols being the same as in the previous cases. The relation between λ and α is

$$\lambda = 8\pi^2 m_0 \alpha k/h^2. \tag{14}$$

The usual computations yield

$$\epsilon_1 = 0, \epsilon_2 = -h^4(30n^2 + 30n + 11)/4096\pi^8 m_0^4 \nu_0^4 \tag{15}$$

and

$$\varphi_1 = h^2 \sqrt{2m_0\nu_0 h}\{\sqrt{n(n-1)(n-2)}\,\psi_{n-3} + 9n^{3/2}\psi_{n-1} - 9(n+1)^{3/2}\psi_{n+1} - \sqrt{(n+3)(n+2)(n+1)}\,\psi_{n+3}\}/768\pi^5 m_0^3 \nu_0^3, \tag{16}$$

where, for example,

$$\psi_{n+3} = e^{-x^2/2a^2} H_{n+3}(x/a)/N_{n+3} \tag{17}$$

Thus to this approximation

$$E = h\nu_0(n + 1/2) - 15h^2\alpha^2(n^2 + n + 11/30)/16\pi^2 m_0, \tag{18}$$

a result agreeing with that obtained by other methods.[5]

Using the first approximation for the eigenfunctions and neglecting terms beyond α^2, it is found that

$$\bar{x} = 3\alpha h(2n + 1)/8\pi^2 m_0 \nu_0, \tag{19}$$

and

$$\overline{(\Delta p_x)^2}\,\overline{(\Delta x)^2} = \{h(n + 1/2)/2\pi\}^2 + \alpha^2 h^3(328n^4 + 656n^3 + 650n^2 + 322n + 123)/2304\pi^4 m_0 \nu_0. \tag{20}$$

It is interesting to consider the effect of the perturbation on the indeterminacy relation. Jevons[6] gives experimental data indicating that if we express E in powers of $n + 1/2$, the ratio of the coefficients of the first and second powers is of the order of 100:1. Since $n^2 + n + 11/30$ is approximately $(n + 1/2)^2$, we may make use of this empirical rule. The following table gives an idea of the results found.

$\pi^2\overline{(\Delta p_x)^2}\,\overline{(\Delta x)^2}/h^2$

n	HARMONIC OSCILLATOR	ANHARMONIC OSCILLATOR
0	0.063	0.063
1	0.562	0.562
2	1.56	1.88
10	27.6	417.

We notice that the effect of the perturbation is to accelerate greatly the increase in the value of the indeterminacy as n increases. The possibility that the approximations made above may not be valid when n becomes

large would merely mean that the values for the anharmonic oscillator would become still larger than those given in the above table, thus not affecting the main conclusion.

The author wishes to express his most sincere appreciation of the guidance, assistance and encouragement of Professor R. B. Lindsay. Much of this work was done while the author was the recipient of a Brown University Fellowship.

[1] Remak, *Zeits. f. Phys.*, **69**, 332 (1931).

[2] Loc. cit.

[3] Condon and Morse, *Quantum Mechanics*, p. 122.

[4] A more general potential function was considered by Dunham, *Physical Rev.*, **41**, 721 (1932).

[5] Birtwistle, *The New Quantum Mechanics*, p. 99.

[6] Jevons, *Band Spectra of Diatomic Molecules*.

MODIFIED RITZ METHOD

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1. The Ritz variation method[1] enables one to approximate to the lowest energy level and wave function of that level in characteristic value problems of the form

$$H\psi_n = W_n\psi_n,\ W_0 \leq W_1 \leq W_2 \ldots \qquad (1)$$

where W_n is the energy level of the state ψ_n of the operator H. The ψ_n are supposed to form a complete orthogonal set. It is based upon the fact that given any arbitary function ξ subject to the restriction $\int \xi\xi^* d\tau = 1$, then $I_1 = \int \xi H\xi^* d\tau \geq W_0$. Thus, the less we make I_1 by so choosing ξ, the closer is I_1 to W_0. MacDonald[2] has shown that when applied to a linear combination of n independent functions, this method gives an upper bound to the n'th excited energy level.

This method, however, suffers from the defect that only an upper bound is given. Thus, while it enables us to better the function, it gives us no information as to how accurate the function really is.

We are going to show in what follows that if W_j is the j'th excited energy level to which I_1 lies closest, then

$$\sqrt{I_2 - I_1^2} + I_1 \geq W_j \geq I_1 - \sqrt{I_2 - I_1^2}$$

where I_1 is as defined above, and $I_2 = \int (H\xi)^2 d\tau$.

2. Consider the function

$$I_2^1 = \int [(H - V)\xi]^2 d\tau , \quad \int \xi^2 d\tau = 1 \tag{2}$$

where V is an arbitrary constant, and let ξ for convenience, be taken as real. Expanding ξ in terms of the orthogonal functions ψ_n, we have $\xi = \Sigma a_n \psi_n$ and $\Sigma a_n^2 = 1$. Inserting this expansion into Eq. (2), and applying Eq. (1) we obtain

$$I_2^1 = \Sigma a_n^2 (W_n - V)^2 \geqslant 0. \tag{3}$$

If we select W_j such that $(W_j - V)^2 \leq (W_n - V)^2$ for all n, or in geometrical language, select that energy level to which V lies closest, then

$$I_2^1 \geqslant (W_j - V)^2. \tag{4}$$

This is easily seen because

$$I_2^1 = (W_j - V)^2 + \Sigma a_n^2 [(W_n - V)^2 - (W_j - V)^2] \tag{5}$$

and the second quantity on the right-hand side is positive.

We have two cases to consider.

Case I	$W_j - V \geqslant 0$
Then from Eq. (4)	$\sqrt{I_2^1} \geqslant W_j - V$
So that	$\sqrt{I_2^1} + V \geqslant W_j \geqslant V$
Case II	$W_j - V \leq 0$
Then from Eq. (4)	$\sqrt{I_2^1} \geqslant - W_j + V$
So that	$V \geqslant W_j \geqslant V - \sqrt{I_2^1}$.

Since V must be either as in Case I or II, we must have

$$\sqrt{I_2^1} + V \geqslant W_j \geqslant V - \sqrt{I_2^1}. \tag{6}$$

We have thus arrived at an upper and lower bound for W_j. It would be well to make the meaning of Eq. (6) quite clear. As has been stated, the constant V is completely arbitrary. The order of the energy level W_j, on the other hand, is determined by V since it is that energy level to which V lies closest. However, this method does not reveal the order of the energy level to which V lies closest, so that essentially Eq. (6) tells us that its upper and lower bounds enclose some one of the energy levels.

We see from Eq. (6) that the term $\sqrt{I_2^1}$ represents the maximum error of the approximation to one of the energy levels. Obviously, we wish to make this error as small as possible. This can be done in the first place by a suitable choice of V. Let us choose V so as to make I_2^1 a minimum.

Now $I_2^1 = I_2 - 2VI_1 + V^2$, where $I_2 = \int (H\xi)^2 d\tau$. Obtaining the minimum, therefore, we find that $V = I_1$ and $I_2^1 = I_2 - I_1^2$. Thus we finally obtain

$$\sqrt{I_2 - I_1^2} + I_1 \geqslant W_j \geqslant I_1 - \sqrt{I_2 - I_1^2}. \qquad (7)$$

No confusion should arise from the use of W_j in both Eq. (6) and Eq. (7). The energy level in Eq. (7) is the one to which I_1 lies closest, and this is not necessarily the one to which V lies closest. We can also say the same thing about Eq. (7) that we said about Eq. (6); namely, we do not know the order of the energy level to which I_1 lies closests, so that we can only say that the upper and lower bounds of Eq. (7) enclose some one of the energy levels.

Eq. (7) can be used for two purposes. First, it can be used to give the maximum error from the nearest energy level when I_1 is determined by any method, and in particular, the Ritz method.

Second, it can be used as a method of finding an energy level. The term $\sqrt{I_2 - I_1^2}$ represents the maximum error for one of the energy levels. Naturally we would like to make this error as small as possible. This we can do by varying ξ. The rule then is: Choose ξ so that $\sqrt{I_2 - I_1^2}$ is minimized.

Theoretically, at least, one can make this error arbitrarily small, for in the limiting case that ξ is a correct solution of Eq. (1), this term clearly vanishes. This constitutes another reason for choosing $V = I_1$. Furthermore, the term $\sqrt{I_2 - I_1^2}$ furnishes us with a criterion of the "best" approximation. On the other hand, the nearness of I_1 to a true energy level does not necessarily furnish us with such a criterion. For example, I_1 may be equal to some true energy level, yet ξ need not necessarily be a true solution at all.

3. Previously,[3] we have used the function $\int \xi H^2 \xi d\tau$ in attempting to improve the Ritz method. This function is formally equal to the I_2 of this note, as can be easily verified by substituting the expansion for ξ, and making use of orthogonality of ψ_n. However, it was found in some special cases that I_2 was not equal to this function, probably because of lack of convergence of the infinite series involved. Therefore, a discussion of the validity of Eq. (4) is necessary. While we cannot do this for a completely arbitrary operator H, it is not difficult to do so when H is of the form

$$H = \nabla^2 + U \qquad (8)$$

where U is a general potential function. In this case one finds that the Eulerian equation of the variation problem

$$\delta \int (H\psi)^2 d\tau = 0\,, \qquad \int \psi^2 d\tau = 1 \qquad (9)$$

is the equation obtained from Eq. (1) by operating with H twice; namely

$$H^2\psi = \lambda\psi \qquad (10)$$

where λ is the Lagrange multiplier, and which must be equal to W^2. Therefore, we can conclude similarly to the Ritz method that $I_2 \geqslant W_j^2$, where W_j is the smallest energy level in absolute value of Eq. (1). If we subtract a constant V from Eq. (8), and apply this result we get Eq. (4).

[1] Courant-Hilbert, *Meth. Math. Phys.*, 149, Springer (1930).

[2] J. K. L. MacDonald, *Phys. Rev.*, **43**, 830 (1933); see also C. Eckart, *Ibid.*, **36**, 878 (1930).

[3] D. H. Weinstein, *Ibid.*, **40**, 797 (1932); **41**, 839 (1932).

A DISCRIMINATION WITHOUT PREVIOUS CONDITIONING

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Communicated August 6, 1934

If two stimuli, $S_{AB..}$ and $S_{AB..L..}$, differing with respect to the property L, are conditioned to elicit a common response R, a discrimination may be established by extinguishing the response to one of them (say, to $S_{AB..}$) while continuing to reinforce the response to the other. The extinction interferes with the reinforcement (and vice versa) through "induction," to an extent determined presumably by the degree of community of properties of the stimuli. The discrimination curve obtained with the present method[1] is an extinction curve resulting from (1) the previous reinforcement of the extinguished reflex ($S_{AB..} - R$), (2) the previous reinforcement of ($S_{AB..L..} - R$), affecting ($S_{AB..} - R$) through induction and (3) the concurrent inductive effect upon ($S_{AB..} - R$) of the periodic reconditioning of ($S_{AB..L..} - R$). If, in a special case, ($S_{AB..} - R$) has not been conditioned before the beginning of the discrimination, the resulting curve will be composed of responses from only the last two of these sources. This case has been reported.[2] The present experiment reduces the problem a step further by beginning the discrimination before either reflex has been conditioned.

The apparatus has been described elsewhere.[1] It consists of a lever, which may be pressed downward by a white rat, the movement of which releases a pellet of food from a magazine into a tray at the will of the experimenter. A light directly above the lever may be turned on as a discriminatory stimulus. All responses to the lever are recorded automatically in the form of graphs showing number of responses vs. time.

In this apparatus eight rats were given the usual training prior to conditioning, which served to adapt the animals to the experimental box and to set up discriminatory responses to the tray based upon the sound of the food-magazine. The response to $S_{AB..L..}$ was then periodically reinforced, and all resulting responses to $S_{AB..}$ were extinguished.

A typical result is shown in figure 1, which gives records for six successive days for one rat. At the release of the rat on the first day the light was on. An investigatory response to the lever occurred quickly and was reinforced. The light and food-magazine were then disconnected. According to earlier investigations[3] some conditioning of $(S_{AB..L..} - R)$

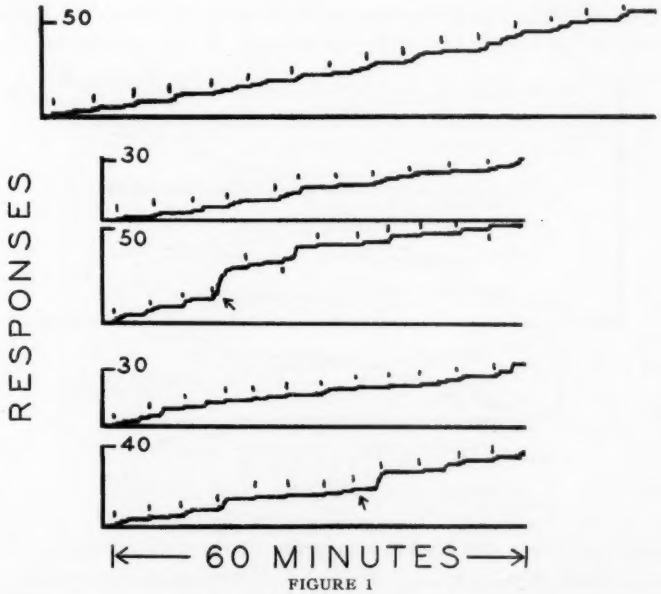

FIGURE 1

very probably occurred. If there were an inductive effect upon $(S_{AB..} - R)$, an extinction curve should follow in the absence of the light. Actually only two responses were forthcoming during the next five minutes, and it is difficult to say whether they show induction or are investigatory responses also. When the light was turned on again (at the second dot over the first record in the figure), the rat responded after 39 seconds. Both light and magazine were then turned off, and two more responses in the dark occurred during the next five minutes. This procedure was repeated for $1^1/_2$ hours, with the result shown in the figure. By the seventh reinforcement the latency of $(S_{AB..L..} - R)$ had reached a more or less

stable value of about 20 secs., which was maintained throughout the rest of the experiment. The rate of conditioning to be inferred from this drop is much lower than that previously reported. It obviously shows interference from the extinction of $(S_{AB..}-R)$.

The strength of $(S_{AB..}-R)$, on the other hand, increases slightly through induction during the first $1^1/_2$ hours to yield a rate of responding of about four responses per reinforcement. On the following day it has dropped to an average of about two responses again, and continues to decline as the discrimination becomes more effective. On the third day an extinction curve appeared spontaneously after the fourth reinforcement, and a similar effect was observed in two other cases. It is as if the response at this point were made in the absence of the light—as if the reinforcement were applied to $(S_{AB..}-R)$. We can account for this anomalous record

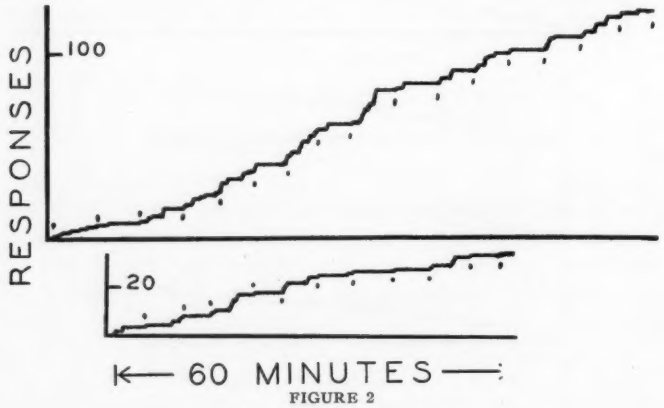

FIGURE 2

by supposing either that the rat was in the course of a response to $S_{AB..}$ when the light was turned on, or that it responded to the lever in the presence of the light but in such a way that the light was not an effective part of the total stimulus. In support of this explanation fairly similar curves can be obtained by allowing one reinforcement to take place in the dark. This was done at the arrows in the last records of figures 1 and 3. In contrast with the spontaneous curves the extinction begins in these two cases only after a delay of approximately three minutes.

Whatever the explanation of this effect may be, it does not seriously disturb the present conclusion, which is that, if a discrimination is begun before either member has been conditioned, the inductive effect may be very slight. Figure 1 is typical of six out of the eight cases. Of the six the greatest induction was observed in the case of figure 2. (This

series was broken on the third day through a technical fault.) The least induction was observed in the two remaining cases, in which an induction in the opposite direction resulted in the early disappearance of all responses. In figure 3 a similar reciprocal induction nearly brought the series to an end in the last part of the second record, where the strength of $(S_{AB..L..} - R)$ (as measured from its latency) fell severely. On the following day there was an adequate recovery, although the latency for the whole series was consistently high, averaging 41.0 secs. as against 15.5 for the other five rats. The average latency of 19.7 secs. for all six rats is approximately three times the latency observed with previous methods,[1] and indicates that with this method the extinction of $(S_{AB..} - R)$ has in general a more marked effect upon the strength of $(S_{AB..L..} - R)$.

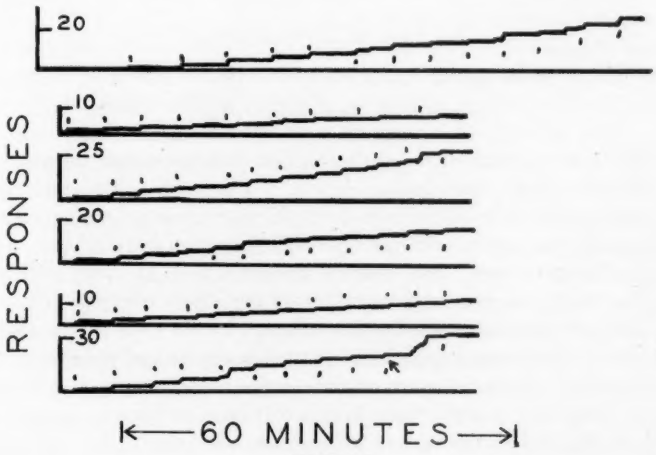

FIGURE 3

The positive induction follows a course to be expected on the basis of previous results. The typical curve for the total series is a protracted S, although the positive acceleration at the beginning may be slight. The greatest slope is never very high, and the subsequent decline is of the sort previously observed.

To control for any unconditioned difference in rate of responding in the presence and absence of the light, half of the above cases were actually of the opposite sort. The experiment in figure 1 was as described, but in figures 2 and 3 $(S_{AB..L..} - R)$ was extinguished and $(S_{AB..} - R)$ reinforced. The two cases in which the rate dropped to zero under negative induction were of the light-on-at-reinforcement type, and this agrees well with a previous report of a depressive effect of the light.[2] Eight cases

are not enough to establish the complete indifference of this condition for the present result, but it is apparent that no very great effect is felt.

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[1] "The Rate of Establishment of a Discrimination," *J. Gen. Psychol.*, **9**, 302–350 (1933).

[2] "A Discrimination Based upon a Change in the Properties of a Stimulus," *Ibid.* (in press).

[3] "On the Rate of Formation of a Conditioned Reflex," *Ibid.*, **7**, 274–286 (1932); and " 'Resistance to Extinction' in the Process of Conditioning," *Ibid.*, **9**, 420–429 (1933).

EXPERIMENTS ON EARLY DEVELOPING STAGES OF FUNDULUS

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Hitherto the ultimate fate of the cells in Fundulus was thought to be established during gastrulation (Lewis,[1,2] Hoadley[3]). The present investigation presents results obtained from explantation and transplantation experiments which give evidence that differentiation is controlled in part by inductive processes. Blastoderms separated from the yolk were cultivated to study the movements and the differentiation of the cells when freed from the mechanical influences afforded by the yolk. Transplantations of materials from the dorsal lip of the blastopore and from the embryonic shield were made to embryonic and extra-embryonic regions of shield stages; the donors of grafts were studied for defective parts. Experiments on Perch eggs (Oppenheimer[4]) have shown that dorsal lip transplants induce embryo-formation, and that grafts of cells from gastrulating stages continue to differentiate when implanted upon the yolk-sac epithelium or in the embryonic body of host embryos.

The explanted blastoderms were separated from the yolk and periblast between the one and the one hundred and twenty-eight cell stage. Morgan[5] secured perfect embryos after one-half to two-thirds of the yolk was removed, but failed to obtain further development if the yolk was reduced to the same size as the blastoderm. The isolated blastoderms in my experiments form spheres; cleavage proceeds at the normal rate but the cleavage planes are in different relationship to each other than those of the blastoderm cleaving on the yolk. After about eighteen hours a large central cavity develops within the round mass of cells. Slightly later a thin-walled vesicle with an epithelium one cell in thickness arises at one pole; at the other pole the remaining cells form a solid mass.

When the cells composing the generalized cell mass have migrated and are aggregated into smaller groups, further cellular differentiation frequently occurs. Chromatophores appear and embryonic structures are recognizable. In some cases well-formed embryos develop. One had well-differentiated fore-, mid- and hindbrain, eyes and ears, a row of seven somites, chromatophores and a beating heart. In this embryo the thin-walled vesicle described above occupied the position normally filled by the yolk. The yolk-sac epithelium is a self-differentiating structure. Histological study of the explants where embryo-formation is imperfect will be necessary to show what tissues and organs have differentiated and to what extent the process is effected by interactions of the materials from different germ-layers.

The differentiation to embryos and embryonic structures with the exception of the yolk-sac epithelium occurs in blastoderms isolated at the thirty-two cell stage and later. The blastoderms isolated at younger stages differentiate the hollow vesicle only. In the early blastoderms where no membrane separates cells from yolk a considerable amount of protoplasm exudes from the cells when first removed from the yolk. The loss is less significant in thirty-two celled and older blastoderms; the central cells have then separated from the periblast and only the marginal cells are in continuity with it. The close correlation of the incomplete development of blastoderms with the greater loss of protoplasm suggests that materials necessary for the subsequent completion of differentiation are located ventrally in the cleaving blastoderm. At present an alternative explanation must be recognized, since such materials may be added to the blastoderm from the periblast before the thirty-two cell stage.

Dorsal lip grafts induce embryo-formation when transplanted into either embryonic or extra-embryonic regions. When the embryos are induced from embryonic regions there is a close but not infallible correspondence in level of primary and secondary embryonic structures. The correspondence is less marked in the case of embryos induced by transplants to the germ-ring in extra-embryonic regions. In no case was the induced embryo complete. The induced embryos form as do the normals from embryonic shields. Stained grafts show that self-differentiation of grafts has not been misinterpreted as induction.

When materials from the shield are implanted in embryonic regions the grafts are not absorbed into the host tissue and then re-sorted, but may remain discrete from the host structures. In some cases they are influenced by the host: in one case a graft to the head-region formed an apparent third optic lobe; in another (Perch) a graft in the somite region became contractile striated muscle. Histological study will be required to ascertain to what degree the grafts are affected during differentiation by their relationships to other structures within the embryo.

The materials transplanted from the shield to extra-embryonic regions undergo self-differentiation, cf. Mangold.[6] In at least one case the graft produced more kinds of structure than were lacking in the donor; the donor showed defects in the mesencephalon; the graft differentiated nervous system, heart and striated muscle. Thus the differentiation of cells already in place in the shield is dependent not only on inherent capacities and on the actions of gastrulation, but also upon the developmental inhibitions exerted by surrounding tissues after gastrulation.

Differentiation in Fundulus is thus seen to be affected by influences exerted during and after gastrulation. The study of predetermination in the cells of the blastula is at present incomplete. In most cases defects of the blastula are regulated (cf. Lewis, Hoadley, loc. cit.). In transplantation experiments, when cells are transplanted from blastula to blastula there are no extra structures in the embryo if the grafts have become mechanically homogeneous with the host tissue. Occasionally, however, they maintain their cellular individuality and appear as masses in the embryo. The early appearance of yolk-sac epithelium in the explanted blastoderms, and the difference in behavior of blastoderms isolated early and late in cleavage, clearly show that processes occurring before gastrulation have a decided influence in the control of differentiation.

[1] Lewis, W. H., *Anat. Rec.*, **6**, 1–6 (1912).
[2] Lewis, W. H., *Ibid.*, **6**, 325–333 (1912).
[3] Hoadley, L., *J. Exp. Zoöl.*, **52**, 7–44 (1928).
[4] Oppenheimer, J. M., *Proc. Soc. Exp. Biol. and Med.*, **31**, 1123–1124 (1934).
[5] Morgan, T. H., *Anat. Anz.*, **8**, 803–814 (1893).
[6] Mangold, O., *Naturwiss.*, **19**, 475–476 (1931).